Membrane Protein Structure I

1329-Pos Board B99

Orientation and Dynamics of Synthetic Transbilayer Polypeptides Containing the GpATM Dimerization Motif

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Deuterium NMR spectroscopy was used to study how the positioning of a dimerization motif within a transbilayer polypeptide influences the polypeptides' orientation and dynamics in phospholipid bilayers. Three polypeptide variants comprising glycophorin A transmembrane (GpATM) dimerization motifs incorporated into lysine-terminated poly-leucine-alanine helices were mixed into 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidyl-choline (POPC) multilamellar vesicles. The variants differed in orientation of the motif segment around the helix axis with respect to the peptide ends. Polypeptides were labeled with methyl-deuterated alanines at positions that were identically situated relative to the peptide ends (Ala-20 and Ala-22) and at two additional positions within the motif. An analysis of quadrupole splittings revealed similar tilts for two of the three variants, and similar azimuthal orientations of the peptide ends for all three variants, suggesting that average orientations were dominated by interactions at the bilayer surface. One variant, however, was characterized by a smaller tilt, and fast orientational fluctuations about the helix axis were of significantly smaller amplitude. This may indicate some perturbation of peptide dynamics and conformation by interactions that are sensitive to the motif orientation relative to the peptide ends. For the variant that displayed distinct dynamics, one orientation consistent with observed splittings corresponded to the motif being situated such that its two glycines were particularly accessible to adjacent peptides. These results show that interactions at the membrane surface and peptide-peptide interactions in the bilayer interior can both affect transmembrane peptide orientation and dynamics. They also imply that motif-mediated peptide-peptide interactions may be sensitive to positioning of the motif with respect to those portions of the peptide that interact with the membrane surface.

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Structure of Transmembrane Domain and Dimerization Mechanism of Amyloid Precursor Protein

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Alzheimer's disease (AD) is the most prevalent form of dementia that affects senior people regardless of religious conviction, nationality or color of skin. The central role in understanding the underlying mechanisms of AD plays biosynthesis of amyloid-β peptide (Aβ). Aβ is a product of sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretase. More than half of mutations that cause so called familial forms of AD are situated in membrane domain of APP and in presenilin-1/presenilin-2 that are part of γ-secretase complex and cleavage APP within the membrane. The pathogenic mutations presumably affect the structure and dynamic properties of the APP transmembrane domain by changing its conformational stability, lateral mobility and /or dimerization. In the present study, the structure of right-handed dimer and dynamics properties of the recombinant peptide corresponding to the APP fragment, Gln686-Lys726, which comprises the APP transmembrane domain with an adjacent N-terminal juxtamembrane sequence, were determined. The structure was solved in the membrane mimetic environment composed of dodecylphosphocholine micelles using NMR spectroscopy. The conformation of transmembrane region each subunit does not alter from published monomer structure. Residues GLY709 and ALA713 form classical GxxxA motif for helix-helix interaction. Juxtamembrane part of investigated peptide does not form tight secondary structure. In this research for the first time dimerization mechanism of APP in atomic resolution is described.

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NMR Studies on Membrane Protein OEP16

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Membrane protein structure is continuing to be a topic of interest in the scientific community. However, high resolution structural data of these proteins is difficult to obtain. In this study we attempt to use Nuclear Magnetic Resonance (NMR) to begin investigating the finer structural aspects of membrane protein OEP16. Latest structural information suggest a multimer consisting strongly of alpha helices. The amino acid transporter located on the outer membrane of chloroplast organelles, Outer Envelope Protein 16kDa (OPE16) was expressed recombinantly in *E. coli* BL21 (DE3) cells for use in NMR spectroscopy. Uniform isotopic labeling with ^{13}C and ^{15}N together with 80% perdeuteration allowed for ~90% of the polypeptide/protein backbone assignments to be carried out using the three dimensional experiments HNCO, HN(CA) CO, HNCA, HN(CO)CA, HNCACB, and CBCA(CO)NH using a 600 MHz spectrometer equipped with a cryoprobe. Chemical shift indices were analyzed for secondary structure through TALOS and PECAN algorithms. Together with NOE connectivities these will enable bond-angle restraints to be introduced for calculating a structural model of the protein. In addition we expressed OEP16 with ^{15}N uniform isotope labeling for relaxation measurements of protein structural dynamics. In order to separate the flexible regions from the rigid regions of the protein, TOCSY-HSQC, HSQC-TOCSY, and NOESY-HSQC experiments were performed on ^{15}N-OEP16 to aid in the assignment of side chains and obtain spatial restraints for use in the determination of three dimensional structure. Results of NMR analysis are compared to results of characterizing the protein by dynamic light scattering (DLS) and circular dichroism (CD) spectroscopy. The findings described in this study provide the first steps in the structure determination of OEP16 and will ultimately contribute to the now limited library of membrane protein structures.

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NMR Structure Determination of the Membrane Protein MerF in Bilayers

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A general NMR method for determining the three-dimensional structures of membrane proteins in phospholipid bilayers is illustrated with the backbone structure of the mercury transporter MerF from the bacterial mercury detoxification system. MerF has two hydrophobic trans membrane helices and is responsible for the transport of mercuric ions and methylmercury across the membrane, from MerP in the periplasm to MerA in the cytoplasm. MerA is mercuric reductase, the enzyme that reduces the toxic Hg(II) to Hg(0). The structure determination method applied to membrane proteins combines Oriented Sample (OS) solid-state NMR and Magic Angle Spinning (MAS) solid-state NMR methods to measure angular constraints for structure calculations. The three-dimensional protein structure determined in its native bilayer environment enables the interactions of the mercury-binding cysteines with the metal ions and other proteins to be described.

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Structural Studies of the Outer Membrane Protein Ail from Y. Pestis

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The three Yersinia species that are pathogenic for humans each cause distinctive diseases, ranging from gastroenteritis (Y. pseudotuberculosis, Y. enterocolitica) to plague (Y. pestis), one of the most deadly human infectious diseases, classified as a Category A Biothreat Agent. In all three, host recognition and resistance to the complement system are associated with the bacterial outer membrane protein Ail (Attachment invasion locus) and its interactions with the human host proteins Fn (Fibronectin) and Vn (Vitronectin), which play important functions in adhesion, and C4BP (complement component 4b binding protein), which plays important functions in immunity.

Ail belongs to the Ail/Lom family (pfam PF06316) of outer membrane proteins, whose members share amino acid sequence homology in the membrane-spanning segments, but vary widely in the sequences of the extracellular loops. E. coli OmpX is regarded as the prototypical member of this family because it is the only one for which the three-dimensional structure, a transmembrane eight-stranded β-barrel, has been determined. However, while Ail has marked adhesion/invasion activity and is essential for virulence, OmpX has no identified function and is not essential. The four extracellular loops of Ail have completely different amino acid sequences than those of OmpX and are thought to be responsible for function. Here we present NMR results for Ail reconstituted in lipid bilayers and lipid micelles as well as binding assays aimed at characterizing the interactions of ail with its human host partners.

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Structural Investigations of the Transmembrane Segment of the PDGF Receptor Beta ant the Oncoprotein E5 by Circular Dichroism and NMR

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The Platelet-derived growth factor receptor β (PDGFR) is a member of the receptor-tyrosine-kinase family involved in development. The receptor is